

Patient Fat Biopsies for Chemical Analysis and Liver Biopsies for Ultrastructural Characterization after Exposure to Polychlorinated Dioxins, Furans and PCBs

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A subset of workers was followed after exposure to polychlorinated biphenyls (PCBs), dioxins, and furans in an occupational medicine clinic setting. Patterns of PCBs found in adipose tissue resembled the pattern seen when soot from the incident or Aroclor 1254 was examined by GC-MS. Adipose tissue also revealed levels of hepta- and octachlorinated furans and dioxins as high as 8400 ppt in one repeatedly exposed worker. Control fat from patients with no known exposure to furans or dioxins was usually in the several hundred parts per trillion range for these isomers, but slightly over 2000 ppt in one sample.

Electron microscopic analysis of liver biopsies from three patients who developed mild elevations of hepatic enzymes in their serum revealed morphologic alterations in some ways similar to those seen in animals after feeding experiments with PCBs, dioxins or the Binghamton State Office Building soot. These include pleomorphic mitochondria, giant mitochondria, prominent dense mitochondrial granules, cristae parallel to the long axis of the mitochondria and crystalline structure within same mitochondria as well as lipid droplets in liver cells and slightly dilated smooth endoplasmic reticulum.

Introduction

On February 5, 1981, at 5:30 in the morning, an increase in electrical current to an electrical panel in the Binghamton State Office Building (BSOB) in Binghamton, NY, led to an accident whereby fluid containing 65% polychlorinated biphenyls (PCBs) (Aroclor 1254) and 35% tri- and tetrachlorinated benzene leaked from an electrical transformer and contaminated the building. Dioxins and furans as well as biphenylenes were formed during a pyrolytic conversion of the chlorinated

benzenes and PCBs. Soot containing these chemicals contaminated the entire building, which has not been used from that date to the present (February 1985) (1,2).

During the first few weeks after the incident, instances of workers being contaminated by and reacting to these chemicals were noted. Transient skin rashes as well as frequent headaches, insomnia and rare instances of peripheral nerve problems were also seen initially. Elevated serum enzymes consistent with liver damage not previously seen and not clinically attributable to an etiology other than the toxic chemical exposure were noted in some patients.

This paper reports on the results of fat biopsies performed on a subset of patients who were exposed to dioxins and related chemicals and analyzed for dioxins, furans, and PCBs and of PCB patterns with respect to higher chlorinated PCBs, the hexa- and heptachlorinated PCBs, which are thought to remain in adipose tissue longer than the lesser chlorinated PCBs.

Three patients with liver pathology with onset dating after their exposure to the toxic chemical exposure, and

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for whom no other etiology could be established, had percutaneous needle biopsies and both light and electron microscopic examination performed. Clinically, after examination by an internist, a gastroenterologist and a preventive medicine/occupational medicine specialist working primarily on toxic chemical exposures, the three patients were felt to have liver damage caused by the chemicals in question. Because the liver, not the skin as is commonly thought, is the target organ of dioxins and related compounds in all animal species studied to date, the liver was of especial interest.

Animal toxicologic studies utilizing PCBs and dioxins has revealed certain morphological lesions in hepatic parenchymal cells at an ultrastructural level (3-9). The BSOB soot has been fed to guinea pigs (10,11) at 1, 10, 100 or 500 mg soot/kg levels, and these oral toxicity studies showed certain untrastructural lesions similar to those described previously for PCBs or dioxins. These included lipid droplets, mitochondrial alterations in shape and also cristae lined up parallel to the long axis, concentric membrane arrays, proliferated smooth endoplasmic reticulum, decreased rough endoplasmic reticulum and hepatocyte enlargement at all experimental doses given. Necrosis of cells and occasional polymorphonuclear or binucleated cells were also seen. Guzelian (12), dealing with needle biopsies of human liver after Kepone or chlordecone poisoning, has also noted ultrastructural lesions similar in some ways to PCB or dioxin lesions in animals, especially with respect to mitochondria, and crystalline structure within mitochondria as well as changes in endoplasmic reticulum.

Methods

For light and electron microscopy, liver tissue was obtained by percutaneous needle biopsy from the upper right quadrant of the liver. Local anesthesia was used by the gastroenterologist obtaining the tissue and patients were observed as outpatients for half a day after biopsy with blood pressure and pulse monitored to detect possible hemorrhage. Complications were not observed in any patients. For light microscopy, tissue was placed in buffered formaldehyde and then embedded in paraffin. It was then sectioned at about 5 μ m and stained with hematoxylin and eosin or a reticulum stain. For electron microscopy the tissue was cut with razor blades into 1-mm pieces while in 2.5% phosphate-buffered glutaraldehyde, at 4°C, with a pH about 7.2 and a milliosmolarity between 300 and 400 milliosmoles. Specimens were fixed between 2 and 4 hr in glutaraldehyde and then washed in the usual fashion in phosphate buffer. Specimens were then processed in 2% phosphate-buffered osmium tetroxide, dehydrated through a series of graded ethanol solutions, placed in propylene oxide and then transferred to Epon. After curing in an oven at 60°C for 48 hr the specimens were sectioned with a diamond knife at a thickness of about 800 Å and placed on copper grids. Sections were stained with uranium and lead salts. A Phillips 201 electron microscope was

used for examination of the tissues at an accelerating voltage of 60 kV. Original magnification of approximately 1,000 \times to 50,000 \times was used to prepare photographic plates.

Extraction and Preliminary Separation of Polychlorinated Dibenzo-*p*-dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) from Adipose Tissue Matrix and Other Residues

In The Brehm Laboratory, Wright State University, Dayton, OH protocol for analysis of the human adipose tissue for tetrachlorodibenzo-*p*-dioxins, an accurately weighed aliquot (typically 5-20 g) of tissue was placed in a clean 125-mL flint glass bottle fitted with a Teflon-lined screw cap, and 10 ng of the internal standard (³⁷Cl⁴-2,3,7,8-TCDD) was added. To the vessel containing the sample 40 mL of 40% (w/v) potassium hydroxide and 20 mL ethanol were added, and, after sealing, the bottle was placed in an oven at a temperature of 100°C for approximately 20 min to initiate saponification. Then the bottle was transferred to a wrist-action shaker and agitated for a period of 16 hr. Following digestion, the digested sample mixture was transferred to a 250-mL flint glass bottle fitted with a Teflon-lined screw cap, using 50 mL 1:1 ethanol-water to rinse the original container. To the mixture was added 40 mL petroleum ether. The sample bottle was placed on the wrist-action shaker for 30 min. The mixture was allowed to stand for a period sufficient for complete separation of aqueous and organic layers to occur and then the aqueous base (bottom) layer was removed and discarded. The organic extract was washed with 50 mL of doubly distilled water by agitating with the extract for 2 min. A sufficient amount of time was allowed for complete separation of the aqueous and organic layers, and the aqueous (bottom) layer was removed and discarded. The organic extract was washed with 50 mL of concentrated sulfuric acid by agitation with the sample for 2 min. Complete layer separation was allowed and the acid (bottom) layer was discarded. The sulfuric acid wash was repeated if the acid layer was visibly colored. Then, the organic extract was washed again with 50 mL of doubly distilled water by agitation for 2 min. Complete layer separation was allowed, and the aqueous (bottom) layer was removed and discarded. The organic extract was dried over sodium sulfate and then quantitatively transferred to a clean test tube. The volume was reduced to incipient dryness using a stream of prepurified nitrogen while maintaining the test tube in a 55°C water bath. A glass Macro-column (20 mm OD \times 230 mm long) was tapered to 6 mm OD on one end. The column was packed with 1.0 g silica, 2.0 g silica containing 33% (w/w) 1 M NaOH, and 1.0 g silica, 4.0 g silica containing 40% (w/w) sulfuric acid, and 2.0 g silica. The previously obtained residue was quantitatively transferred to the column and the

column was eluted with 90 mL hexane. The eluent and concentrate were collected to 1 to 2 mL in a centrifuge tube.

The disposable liquid chromatography column was constructed as follows. A Pyrex 5 mL disposable pipet was cut off at the 2 mL mark, and the lower portion of the pipet was used. The small end was packed with a plug of silanized glass wool. Next, 1 g of Woelm basic alumina previously activated overnight at 600°C in a muffle furnace was added, and the column then placed in a dessicator for 30 min just prior to use. Using a disposable pipet, the concentrate was transferred onto the liquid chromatography column. The centrifuge tube which contained the concentrate was rinsed with two consecutive 0.5-mL portions of 3% CH_2Cl_2 in hexane, and the rinses were transferred to the alumina column. The column was then eluted with 8 mL of 3% (v/v) CH_2Cl_2 in hexane, and the eluent was discarded (taking care not to let the column run dry). The column was then eluted with 15 mL of 50% (v/v) CHCl_3 in hexane and the eluent retained for analysis.

The eluent was concentrated to approximately 1 mL with the use of a stream of prepurified nitrogen as before. The centrifuge tube wall was rinsed with an additional 1 mL of CH_2Cl_2 and re-concentrated. The residue was quantitatively transferred (using methylene chloride) to a 2 mL microreaction vessel. The solution in the microreaction vessel was evaporated almost to dryness as previously, and the walls of the vessel were rinsed with approximately 0.5 mL CH_2Cl_2 , and the contents evaporated just to dryness, and the extract stored in freezer until just prior to GC-MA analysis. Approximately 1 hr before GC-MA (GC-LRMS or GC-HRMS) analysis, the residue was diluted in the microreaction vessel with an appropriate quantity of tridecane. Tridecane was gently swirled in the bottom of the vessel to ensure dissolution of the dioxins.

Reagents and Chemicals

The reagents and chemicals utilized in the procedures outlined above are as follows. Potassium hydroxide, anhydrous sodium sulfate, and sulfuric acid were all Reagent Grade and were obtained from J.T. Baker Chemical Co. or Fisher Scientific Co., Fairlaw, NJ. Hexane, methylene chloride and benzene were "distilled in glass" quality obtained from Burdick and Jackson, Muskegon, MI. Ethanol and petroleum ether (low boiling) were Omnisolv Quality solvents from Matheson, Coleman, and Bell, Cincinnati. Woelm basic alumina (Activity Grade I) was obtained from ICN Pharmaceuticals, Cleveland, OH. Silica (Bio-Sil A) was obtained from Bio-Rad, Richmond, CA. Doubly distilled water was obtained by using the all-glass distillation apparatus in the Brehm Laboratory. Prepurified nitrogen was obtained from Airco, Inc., Montavale, NJ. The 2,3,7,8-TCDD standard was obtained from Dow Chemical Co., Midland, MI, and the $^{37}\text{Cl}_4$ -2,3,7,8-TCDD standard was obtained from KOR Isotopes, Cambridge, MA.

Instrumentation and Experimental Parameters for Analysis of Extracts for TCDD

The sample extracts prepared as described above were analyzed for TCDD by using coupled gas chromatography-mass spectrometry (GC-MS) instrumentation.

A Perkin-Elmer Sigma III gas chromatograph was used, coupled through a custom-fabricated interface including a single-stage glass jet separator to a Kratos MS-25 Mass Spectrometer equipped with a DS-50 SM Data System.

Conditions for the gas chromatograph were as follows: Column, 50M WCOT (OV-101) silica capillary; carrier gas, hydrogen, 30 lb. head pressure; column temperature, isothermal at 190°C for 2 min, programmed at 5°C/min from 190°C to 220°C, hold at 220°C for 17 min, then program at 5°C/min to 230°C, hold at 230°C for 23 min, then program at 5°C/min to 235°C, hold at 235°C for 78 min; interface temperature, 250°C; injector, splitless injection.

Conditions for the mass spectrometer were as follows: ionizing voltage, 70 eV; accelerating voltage, 7 kV; Masses monitored in selected ion monitoring mode were 319.897, 321.894, 256.933, 258.930 (all native 2,3,7,8-TCDD) and 327.885 ($^{37}\text{Cl}_4$ -2,3,7,8-TCDD, internal standard). These results can be confirmed by using capillary column GC-high resolution MS if desired. The sequence of operations is given in Table 1.

Results

Morphological Results: Hepatic Parenchymal Cells

The three patients, A, a 41-year-old male supervising engineer, B, a 35-year-old male maintenance worker and C, a 31-year-old male firefighter, were exposed to the chemical mixture of dioxins, furans, PCBs, chlorinated benzenes, chlorinated naphthalenes, biphenylenes, and biphenyl ethers in the course of their work. Each had been under the care of a different primary care physician, and, in one case, a gastroenterologist, before being referred for evaluation of persistent moderate elevation of previously normal liver enzymes, e.g., SGPT of 80 (normal 2–32), gamma-GTP of 122 (0–42), and complaints of fatigue. Detailed medical evaluation could not detect other conditions such as hepatitis, alcoholism, medications or illicit drug use responsible for the elevation of liver enzymes, gamma GTP, SGOT, or SGPT. Elevated triglyceride levels were also sometimes observed in these and other patients after this exposure. In addition, patient C, a firefighter, was noted to have had serum PCB levels of 21.4 ppb from blood taken 3/12/81, one month after the fire, and a level of 6.0 ppb in blood drawn 11/2/81, suggesting ingestion of PCBs which moved into adipose tissue and elsewhere

Table 1. Sequence of operations in GC-MS (MS-25) analyses of PCDD and PCDF in sample extract

Elapsed time	Event	GC column temp. °C	Temp. prog. rate °C/min	Ions monitored by mass spectrometer	
				<i>m/z</i>	Compounds monitored
0.00	Injection, splitless	190			
1.50	Turn on split valve	190			
2.00	Begin temp. program to 220°C	190	5		
5.00	Open column flow to mass spectrometer	205	5		
5.00	Stop program 3	220			
5.50	Start program 4; sweep = 100 ppm; time on each mass = 0.15 sec	220		258.930 303.902 305.899 319.897 321.894 327.885	Tetra-CDD Tetra-CDF Tetra-CDD Tetra-CDD Tetra-CDD ³⁷ Cl ₄ -Tetra-CDD
23.50	Stop program 4	220			
24.00	Start program 5; sweep = 750 ppm; time on each mass = 0.2 sec	220		337.863 339.860 353.858 355.855	Penta-CDF Penta-CDF Penta-CDD Penta-CDD
25.00	Begin temp. program to 230°C	220	5		
34.50	Stop program 5				
35.00	Start program 6; sweep = 150 ppm; time on each mass = 0.25 sec	230		373.821 375.818 389.816 391.813	Hexa-CDF Hexa-CDF Hexa-CDD Hexa-CDD
50.00	Begin temp. program to 235°C		5		
53.50	Stop program 6	235			
54.00	Start program 7; sweep = 750 ppm; time on each mass = 0.35 sec	235		407.782 409.799 423.777 425.774 431.765	Hepta-CDF Hepta-CDF Hepta-CDD Hepta-CDD ³⁷ Cl ₄ -Hepta-CDD
70.00	Stop program 7				
85.00	Start program 8; sweep = 750 ppm; time on each mass = 0.50 sec	235		441.732 443.740 457.738 471.717	Octa-CDF Octa-CDF Octa-CDD ³⁷ Cl ₈ -Octa-CDD
95.00	Stop program 8	235			
130.00	Return to initial				

in the body and which were partially excreted in the 8 months between the first and second blood samples. Patient A, an engineer who was involved in supervising the cleanup, had PCB levels of 6.3 and 7.3 ppb in blood samples from 2/15/81 and 12/3/81, respectively. This is compatible with an increase in body burden of PCBs from having been inside the contaminated area repeatedly. Clinically, regardless of the laboratory findings, the patient did enter the building unprotected several times during "emergencies" and was exposed at those times. Patient B, a maintenance worker, was in the polluted State Office Building but also, as a City of Binghamton worker, was in areas of the basement in Binghamton City Hall which were contaminated during the incident of February 5, 1981, but also was contaminated when parts of City Hall were used for the cleanup as a staging area during February 1981. His serum PCB

levels were 4.0 ppb on 10/14/82 and 5.97 on 11/16/82, consistent with repeated exposures after the original incident (as well as possible earlier contamination) as from the contaminated City Hall.

As noted before, (3-11), previous investigators using animals in feeding studies with PCBs or dioxins found a number of characteristic ultrastructural alterations. Lipid droplets, concentric membrane arrays, stacked membranes, increased smooth and increased or decreased rough endoplasmic reticulum, and mitochondrial alterations in shape and also arrangement of cristae mitochondriales parallel rather than perpendicular to the mitochondrial long axis have been reported. Complicating these findings is the knowledge that until recently it was not possible to measure trace amounts of furans or other isomers which contaminate PCB mixtures. In addition, complicating the findings with re-

spect to the BSOB soot is the fact that the mixture of PCBs, tri- and tetrachlorinated benzenes, dioxins, furans, biphenylenes, biphenyl ethers and chlorinated naphthalenes found along with other still unidentified chemicals, perhaps including vinyl chloride from electrical insulation, which constitutes a unique mixture with possible synergistic, additive, or perhaps even antagonistic properties. Fortunately, the description of Turner and Collins (10), based on the toxicologic investigations of Silkworth and colleagues (11) provides data on the alterations of guinea pig hepatic parenchymal cells from the Binghamton soot. Essentially, their findings show that the usual toxicologic effects caused by dioxins or PCBs on guinea pigs is seen from this particular soot mixture although the furans and other chemicals may be contributing quantitatively and qualitatively to these alterations and pathological changes. They noted hypertrophy of hepatocytes, steatosis, focal necrosis, bile duct proliferation (adenofibrosis), proliferation of smooth endoplasmic reticulum, concentric membrane arrays (CMAs), mitochondrial shape and mitochondrial cristae alterations, decreased rough endoplasmic reticulum, and autophagolysosomes.

The three patients had liver biopsies taken in 1983, two years after initial exposure (Feb. 5, 1981). The light microscopic findings (Figs. 1 and 2) were as follows.

PATIENT A. The liver architecture is normal. Some of the centrilobular cells have several small fat droplets or one large one. In addition, the centrilobular cells have visible but not excessive lipofuscin pigment. A few areas of focal necrosis were noted scattered through the specimen. No fibrosis was noted around the central vein (terminal hepatic venule) and no Mallory bodies were seen.

PATIENT B. The liver architecture is normal. Some cells contain fat droplets mainly in the centrilobular zone. Mild portal fibrosis is noted.

PATIENT C. The liver architecture is intact. A few central hepatocytes contain fat droplets and mild portal fibrosis is present.

The EM findings (Figs. 3–7) are shown in the figures and summarized as follows.

PATIENT A. The overall appearance of the hepatocytes was normal. All hepatocytes contain fat droplets measuring from 1 μm or less in diameter to more than 20 μm . The plasma membranes and bile canaliculi were normal as were the nuclei and nucleoli. Glycogen was abundant in all cells. Both the rough and smooth forms of the endoplasmic reticulum were normal. Slight prominence of smooth endoplasmic reticulum is sometimes observed. Mitochondria were variable in shape, while usually normal in size. Some were elongated or dumbbell shaped. About one mitochondrion in ten had small clusters of cristae parallel to the outer mitochondrial membranes and rare crystalloids were seen. The mitochondrial dense bodies were twice as large as normal in about half the cells. Occasional breaks were detected in the outer mitochondrial membranes. The number and size of lysosomes varied from cell to cell. Most cells had

some lipolysosomes and autophagic vacuoles. Peroxisomes were normal. A mosaic "staining" was noted on some mitochondria.

PATIENT B. The overall appearance of the hepatocytes was normal. Each cell contained one to several fat droplets from less than 1 μm to more than 10 μm in diameter. Plasma membranes, bile canaliculi, nuclei and nucleoli were normal, and glycogen was abundant. The rough endoplasmic reticulum was prominent and the smooth form normal to somewhat prominent. Numerous giant or megamitochondria with crystalloid formation in the matrix were seen. Some were more than 10 μm long and 5 μm in diameter. Branching forms were also seen. Dense bodies were large and numerous in the mitochondria. Some of the normal sized mitochondria without crystalloid structures had cristae parallel to the mitochondrial long axis. A few membrane breaks were noted in outer membranes. The normal sized mitochondria had larger than normal dense bodies. Peroxisomes were normal.

PATIENT C. The overall appearance of the hepatocytes was normal. Plasma membranes, bile canaliculi, nuclei and nucleoli were normal. Small fat droplets and lipolysosomes 1–2 μm in diameter were numerous in almost all cells. A few cells had larger fat droplets. Glycogen was abundant. The rough endoplasmic reticulum was sometimes dilated and increased in amount. Smooth ER was sometimes prominent. The mitochondria were normal in size and somewhat varied in shape with a few elongated and dumbbell forms and a few with narrow projections. About one mitochondrion in ten had one or more cristae running parallel to the long axis of the mitochondrion. A few outer membrane breaks were seen. Some of the lysosomes were adjacent to or had incorporated what appeared to be fat droplets from which most of the fat had been removed. Peroxisomes were normal. Occasional myelinlike bodies were seen, but no classical concentric membrane arrays were noted.

The three cases show changes including mild steatosis, portal fibrosis and focal necrosis. At an electron microscopic level the features in common included fat droplets and mitochondrial cristae changes in a minority of the organelles. Premalignant changes in nucleoli were absent. Pleomorphic mitochondria, mitochondria of giant size and or bizarre shapes with branching structures were seen. Crystalline structures were observed in two of three patients. Cristae within mitochondria were occasionally lined up parallel to the long axis of the mitochondria. Glycogen was abundant; unlike animals, patients were not fasted before biopsy material was obtained. Smooth endoplasmic reticulum was frequently slightly dilated and possibly increased in amount to a small extent. Rough endoplasmic reticulum appeared normal to slightly prominent. Autophagosomes were frequently noted. Lipid droplets were frequently observed. Double nuclei and swollen cells were sometimes observed as were necrotic cells on occasion. Concentric membrane arrays as such were not seen. Myelin bodies were noted. Mild portal fibrosis was sometimes present.

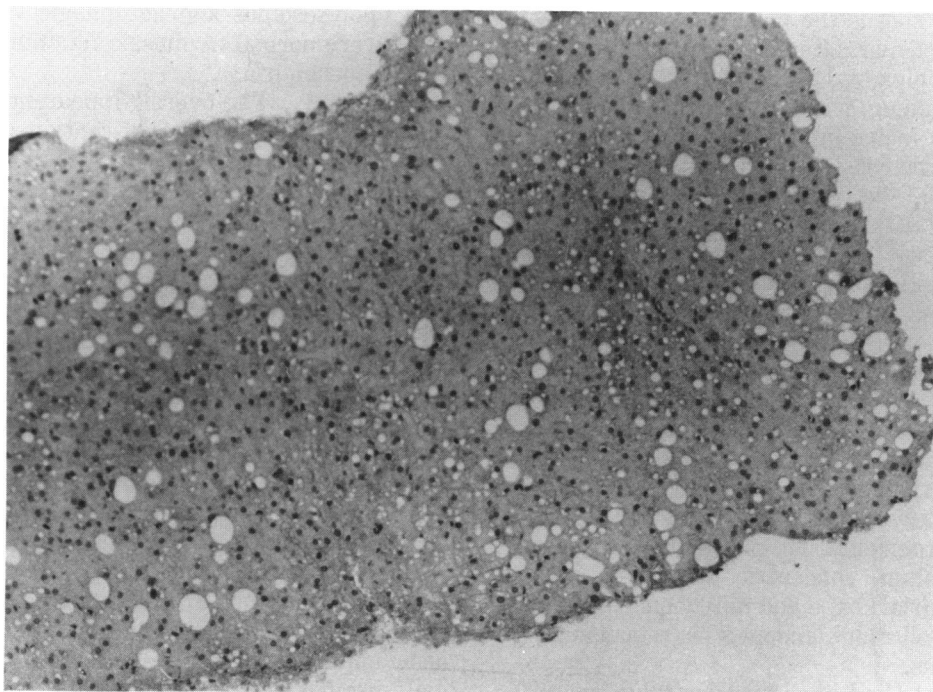


FIGURE 1. Normal architecture with mild fatty changes on light microscopy, with occasional double nuclei and necrotic cells. Little to no evidence of increase in fibrous tissue was found. $\times 690$.

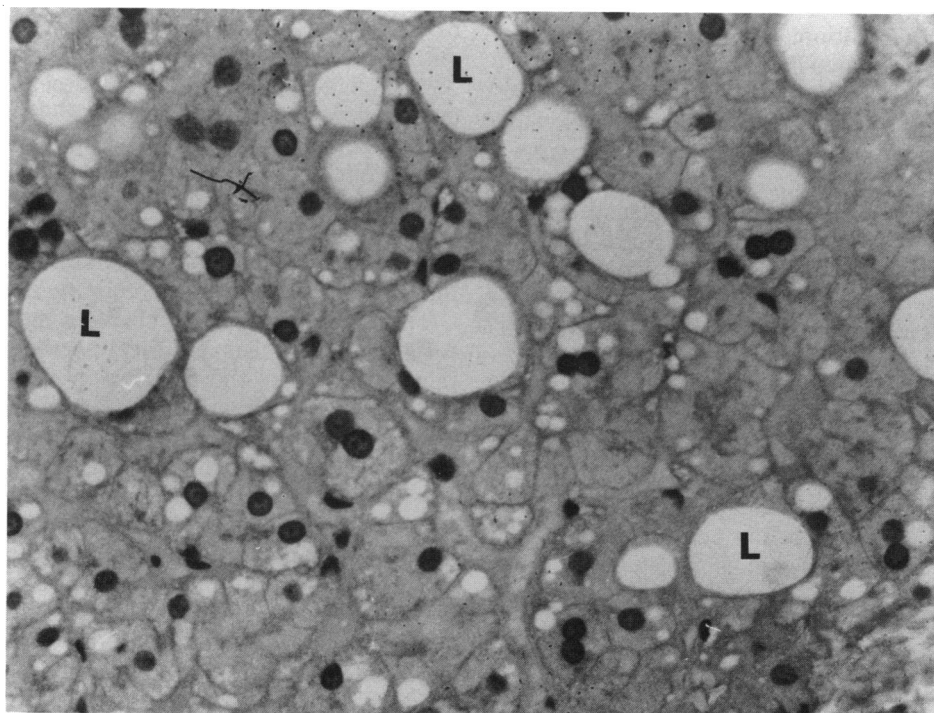


FIGURE 2. Light micrograph at higher magnification shows some of the lipid droplets, occasional double nuclei, and some swelling of cells. $\times 2200$.

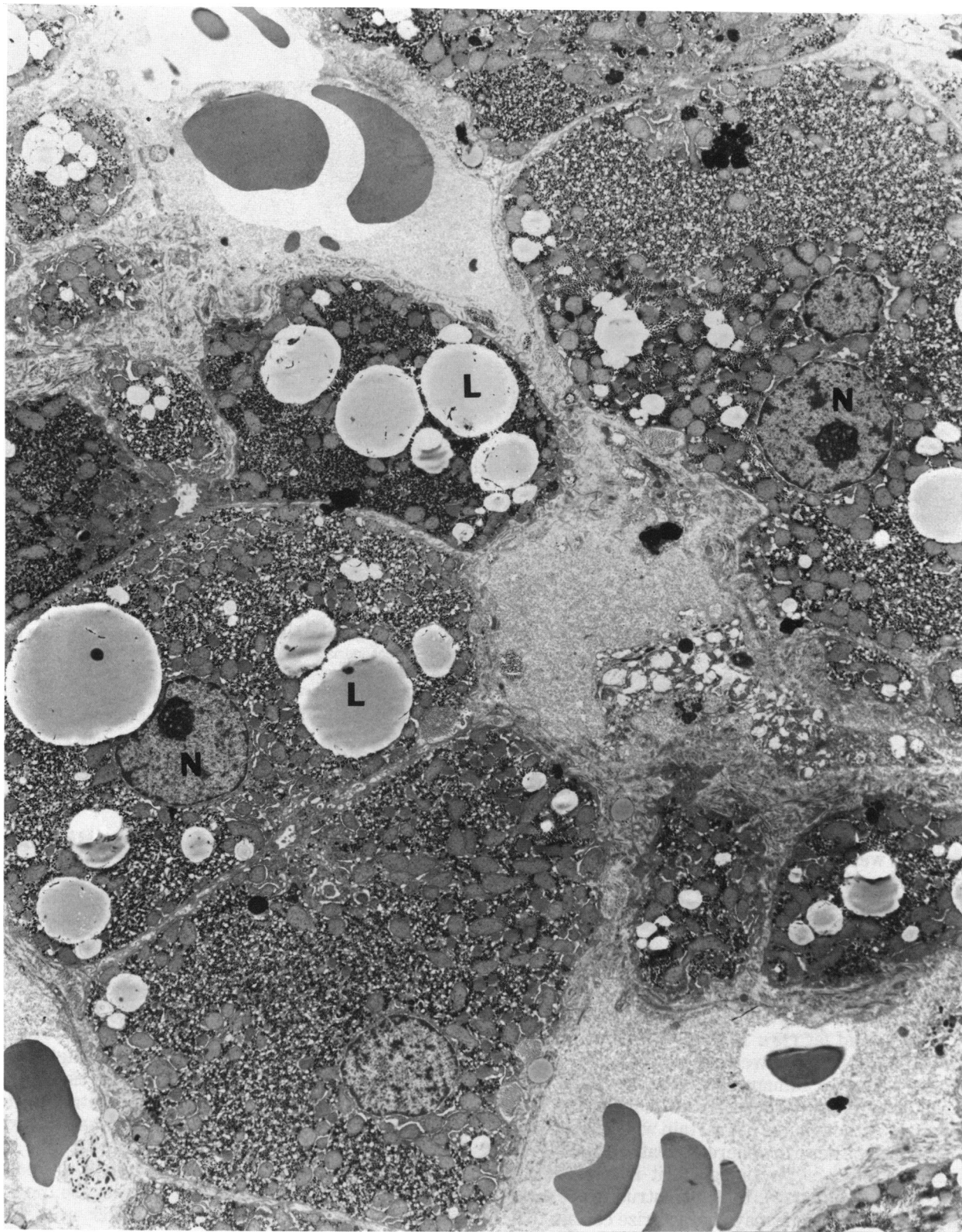


FIGURE 3. Low power electron micrograph, shows lipid droplets (L), abundant glycogen, normal architecture as before, and some suggestion of dilated smooth endoplasmic reticulum in cells on the upper right and lower left. $\times 4410$.

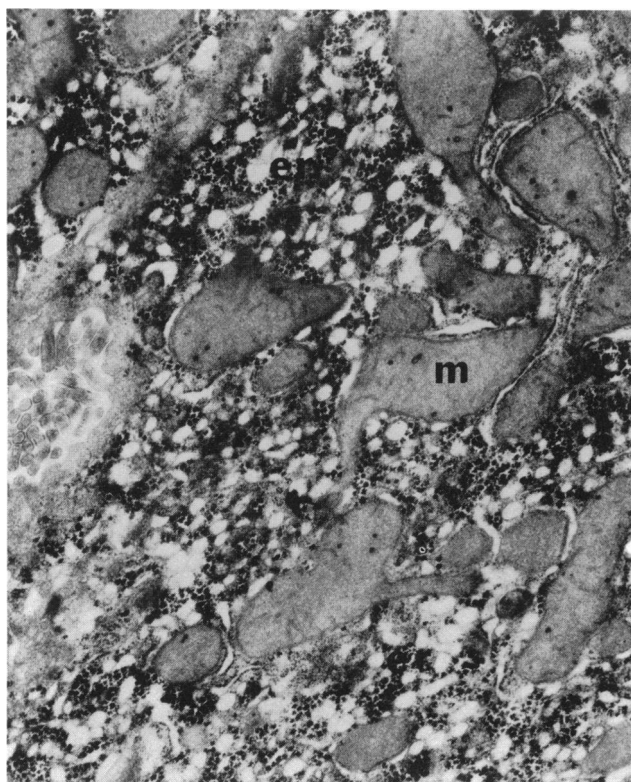


FIGURE 4. Illustrates the slightly dilated smooth endoplasmic reticulum, copious glycogen and the pleomorphic mitochondria (m) seen in all three patients, with bizarre shapes and branching structure. In some cases the mitochondria appear to be pushing against stacks of rough endoplasmic reticulum. $\times 9600$.

It should be noted that in animal studies, except for the Turner and Collins one-dose study (10), frequently large doses of dioxins or related chemicals are given for long periods of time relative to the life span of the animal. The patients were presumably no longer ingesting dioxins and PCBs and related chemicals at the time biopsied in 1983, 2 years after the incident and at least 6 months after the last exposure from the contaminated building. We expect some deposition of these chemicals in liver and fat with slow release over time. We also know from Brandt's work with PCBs in rats that different PCB isomers migrate to different body tissues, such as lung, liver, kidney, and fat (13). For most of the isomers in the Binghamton incident we have no data as to site of tissue deposition in humans, but assume that fat and liver will preferentially house certain isomers and their metabolites. Although it is not certain whether the morphological lesions described here are pathological (other than the necrosis) or physiological, it is important to note, as did Guzelian (12), that ultrastructural characterization of liver lesions may characterize certain toxic chemical exposures in man and may provide us with pathognomonic lesions to assist diagnosis. Guzelian also noted a return to normal hepatic ultrastructural morphology, in the case of chlordecone (Kepone), over time. The lesion appears to constitute a sensitive although



FIGURE 5. Parallel stacked cristae (cr) parallel to the long axis of one mitochondria are seen with normal cristae and prominent dense granules in another mitochondria. These stacked cristae are suggestive of earlier work by Kimbrough. $\times 57,540$.

perhaps not specific marker for exposure to these chlorinated xenobiotics.

PCB Pattern Analysis from Fat Biopsies

Wolff et al. (14,15), Masuda (16), Moroni (17) and others have suggested that fat biopsies for PCBs, PBBs and related compounds including DDT and DDE should involve isomer pattern characterization in addition to merely measuring total amount present to estimate exposure as well as exposure to a specific PCB mixture, which could, in theory at least, permit differentiation between exposure to Aroclor 1254, 1242 or 1016. Following on this suggestion and knowing that higher chlorinated PCBs appear to remain in adipose tissue longer than lower chlorinated PCBs which are metabolized and excreted more rapidly in man, we analyzed a mixture of Aroclor 1254 and chlorinated benzenes for the hexa- and heptachlorinated PCBs and obtained the pattern shown in Figure 8. Of particular interest are the doublets or pairs seen between 250 and 350 on the mass spectrogram pattern. In examining the pattern obtained from a BSOB soot sample (Fig. 9) the doublet or pairing is again noted of two smaller peaks between the larger peaks. In Figure 10 prepared from patient C who was exposed to these chemicals when he took part in

extinguishing the fire we see a doublet smaller set of peaks between the higher peaks, suggestive of, or at least consistent with, exposure to the hexa- and heptachlorinated PCBs like those in Aroclor 1254 and also the Binghamton soot. However, Figure 11, prepared from adipose tissue of a non-exposed patient, looks dissimilar to the previous patient, the soot, or the Aroclor 1254 mass spectrogram. Such "fingerprinting" may well be of great usefulness in ruling out exposure to a specific PCB incident, or in suggesting a "fingerprint" consistent with exposure to a specific type of soot pattern. Obviously, time after exposure, kinetics of the isomers, knowledge of the metabolites and their kinetics, tissues of isomer deposition and other related data would all contribute to the potential usefulness of such an approach.

Fat Biopsy Analysis for Dioxin and Furan Isomers

The analysis of human tissue, including blood, milk, urine, hair and fat to directly measure chlorinated organics, metals and other compounds has long been considered useful in clinical medicine. PCB, PBB and DDT fat analysis have long been performed (14-19). In the case of furan and dioxin analysis, recent work suggests that 5 to 15 ppt of 2,3,7,8-TCDD and several hundred

parts per trillion of 7- and 8-chlorinated furans and dioxins may be found in fat tissue in the general population in the U.S. and Canada (20-22). Knowing that we had a group of patients who were recently exposed to dioxins and furans, we analyzed fat from a subset of the patients being followed by the senior author. Fat was taken from the gluteal region where 5 to 10 g were removed as a simple outpatient procedure, under local anesthesia.

The levels found and the levels of sensitivity of the method are shown in Table 2, which illustrates the finding from a Binghamton soot sample and two patients, including the levels of recovery of radioactive-labeled internal standards. Although our approach included an attempt to match isomers found in fat with those in the soot to which the patients had been exposed 1 to 2 years previously, the analysis of multiple isomers served to decrease the level of sensitivity below that which would have existed had we elected to analyze for only one isomer, with our levels of detection as low as 1 ppt for TCDDs in blood and 10 ppt in adipose tissue (Table 3).

The highest level found by us, and to the best of our knowledge, the highest level of dioxin and furan contamination of human adipose tissue reported in the literature to date, was in an engineer who had worked in the contaminated building during the cleanup over an



FIGURE 6. Shows mitochondria with stacked cristae and crystalloid structures as well as a lipid droplet (L). $\times 57,540$.

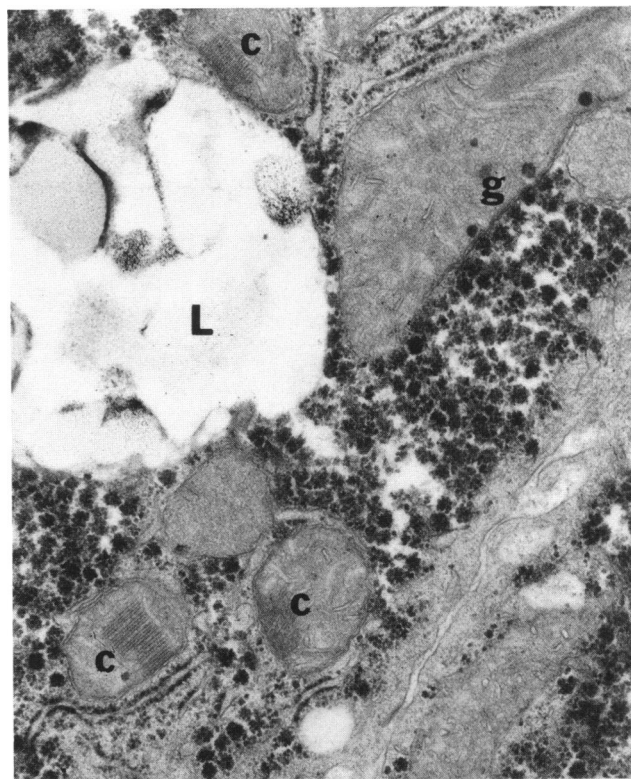


FIGURE 7. Illustrates the mitochondrial crystalline structures (C), a large mitochondrion, and a lipid (L) droplet. Crystalline structures were seen in mitochondria in two of our three patients and described as typical findings by Guzelian (12) in chlordecone-poisoned workers. $\times 35,000$.

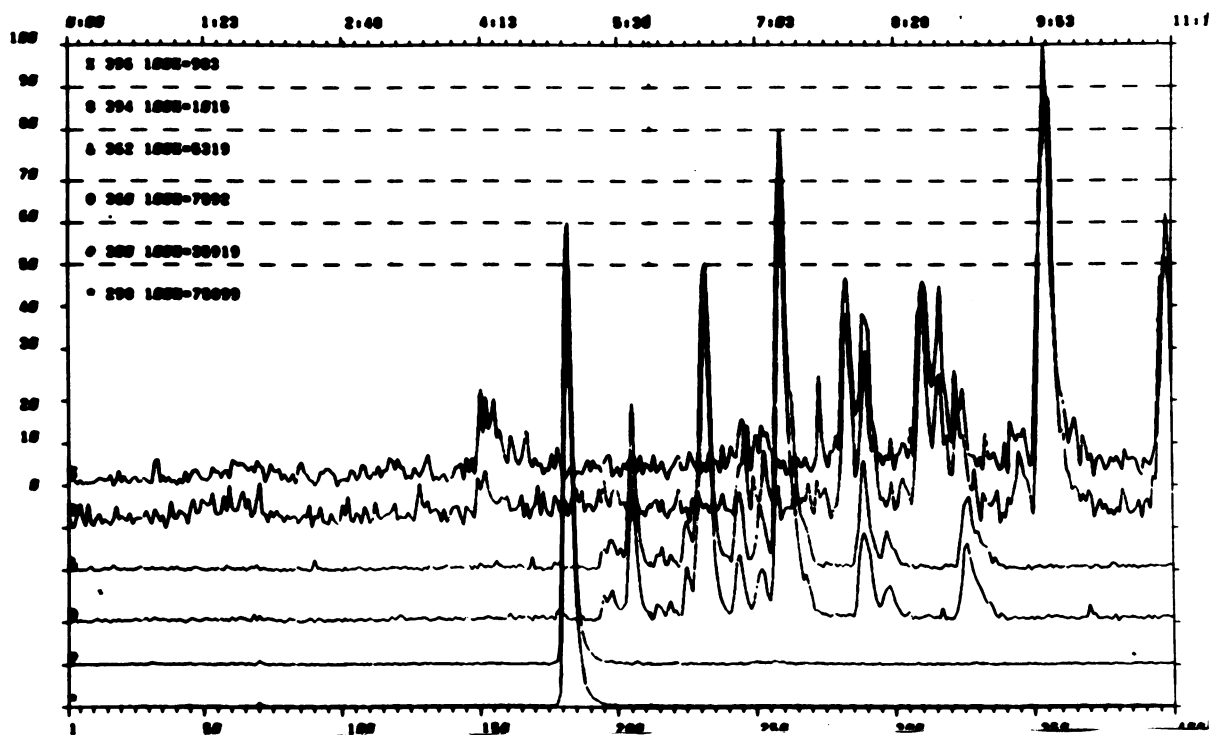


FIGURE 8. Mass spectrogram prepared using Aroclor 1254 and polychlorinated benzenes shows the region where the hexa- and hepta-chlorinated PCBs are found. Moving from right to left a high peak followed by two double lower peaks followed by a higher peak can be seen.

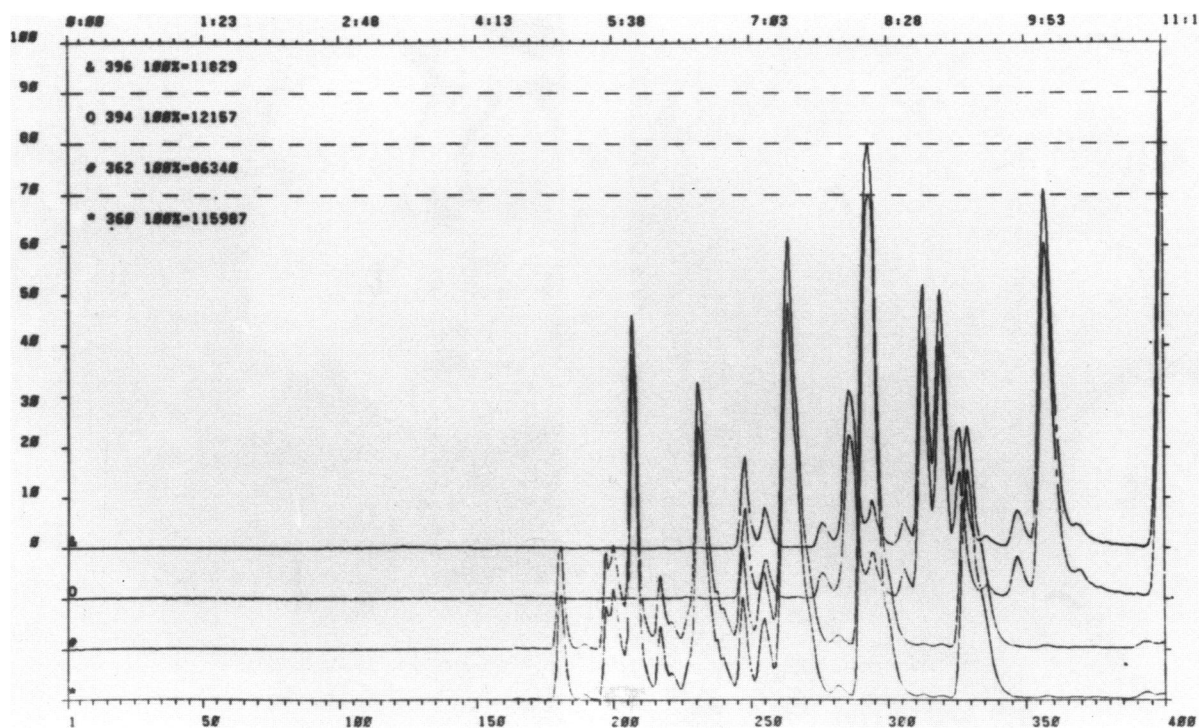


FIGURE 9. Mass spectrogram of the same region as in Fig. 8. It was prepared from an extract of the Binghamton State Office Building soot. A similar pattern can be observed of a high peak on the right-hand side, followed by two lower doublets and then another high peak. These presumably reflect hexa- and hepta-chlorinated PCBs.

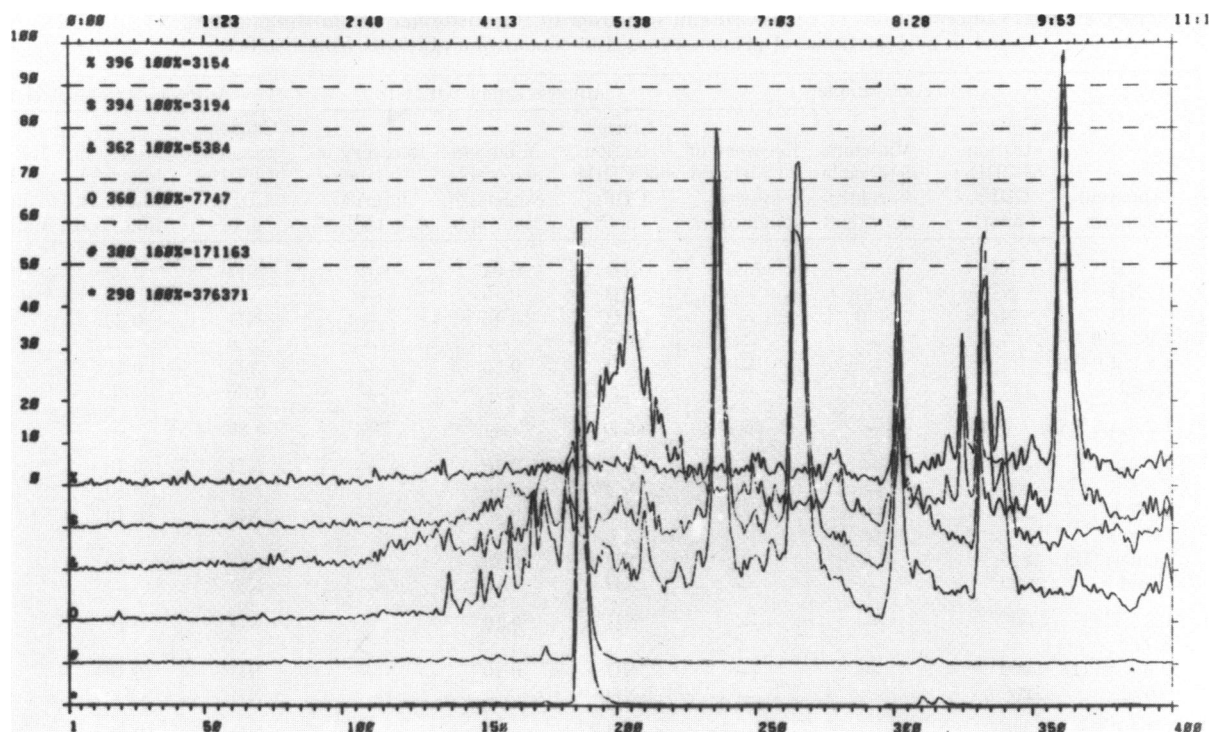


FIGURE 10. Mass spectrogram prepared from an extract from the adipose tissue of a firefighter who assisted in putting out the BSOB fire. This is also in the region of this run where the hexa- and hepta-chlorinated PCBs are seen. The high peaks on the right hand side of this section of the run with the two lower doublets bear a close resemblance to both the Aroclor 1254 and the Binghamton soot patterns.

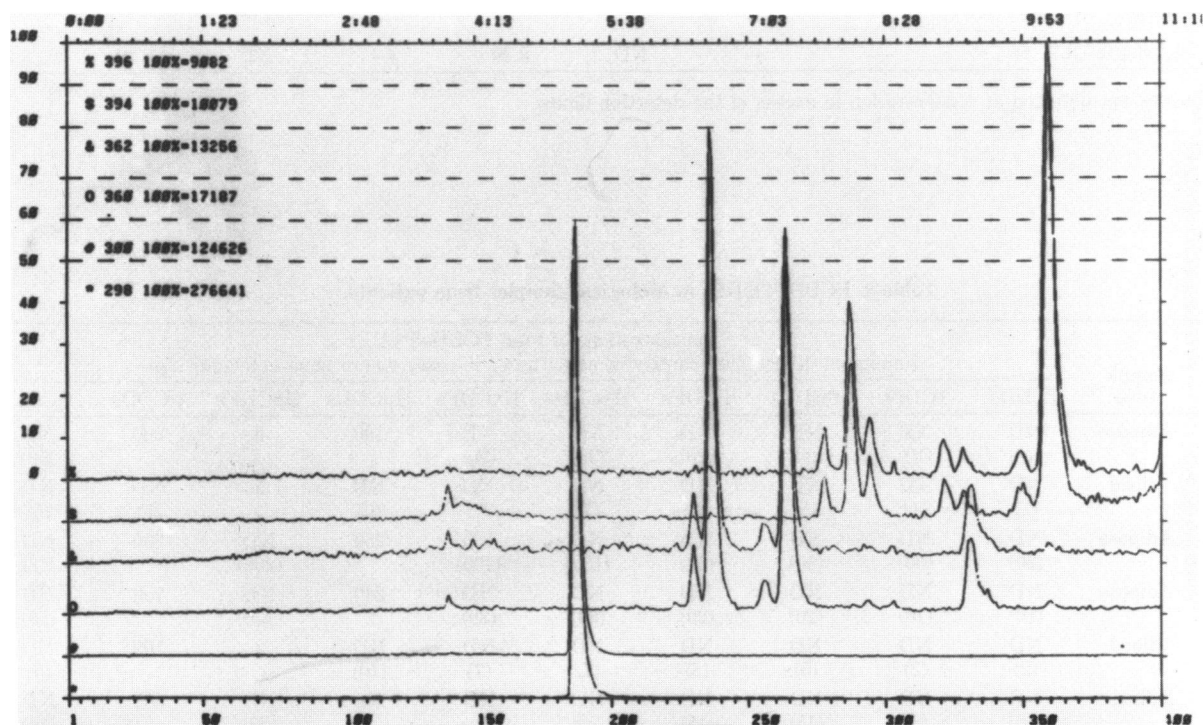


FIGURE 11. Mass spectrogram from the adipose tissue of a control Binghamton patient who had no known exposure to the Binghamton soot. The presence of PCBs of a background pattern is reflected, but the characteristic pattern of the high peaks with the lower doublets between them is absent.

Table 2. Concentrations of CDDs/CDFs in soot from PCB-transformer fire in Binghamton, NY and in adipose tissue of persons exposed to combustion effluents from the fire.

CDD/ CDF	Apparent isomers	Soot sample			Adipose sample-OLM-18			Adipose sample-OLM-15		
		Concen- tration of CDDs/ CDFs, ppm ^a	Minimum detectable concentra- tion, ppm	Recovery of ³⁷ Cl-labeled internal standards, %	Concen- tration of CDDs/ CDFs, ppb ^a	Minimum detectable concentra- tion, ppb	Recovery of ³⁷ Cl-labeled internal standards, %	Concen- tration of CDDs/ CDFs, ppb ^a	Minimum detectable concentra- tion, ppb	Recovery of ¹⁷ Cl-labeled internal standards, %
TCDD	ND	ND	3.0	75%	ND	0.025	80%	ND	0.025	75%
PCDD	ND	ND	2.0	—	ND	0.10	—	ND	0.10	—
HxCDD	ND	ND	3.0	—	ND	0.15	—	ND	0.20	—
HpCDD	1,2,3,4,6,7,8	7.0	—	—	0.20	—	—	0.30	—	—
	1,2,3,4,6,7,9	7.0	—	—	ND	0.15	—	ND	0.15	—
	Total	14	—	—	0.20	—	—	0.30	—	—
OCDD	1,2,3,4,6,7,8,9	5.0	—	65%	0.50	—	70%	0.80	—	60%
TCDF	1,2,4,8	380	—	—	ND	0.10	—	ND	0.10	—
	2,3,6,8	80	—	—	ND	0.10	—	ND	0.10	—
	2,3,7,8	220	—	—	ND	0.10	—	ND	0.10	—
	Other isomers (12)	1240	—	—	ND	0.10	—	ND	0.10	—
	Total	1920	—	—	ND	—	—	ND	—	—
PCDF	1,2,4,7,8	600	—	—	ND	0.10	—	ND	0.10	—
	Other isomers (11)	600	—	—	ND	0.10	—	ND	0.10	—
	Total	1200	—	—	ND	—	—	ND	—	—
HxCDF	1,2,4,6,7,8	220	—	—	ND	0.15	—	ND	0.20	—
	Other isomers (12)	940	—	—	ND	0.15	—	ND	0.20	—
	Total	1200	—	—	ND	—	—	ND	—	—
HpCDF	1,2,3,4,6,8,9	60	—	—	7.7	—	—	3.9	—	—
	Other isomers (3)	345	—	—	ND	0.20	—	ND	0.20	—
	Total	405	—	—	7.7	—	—	3.9	—	—
OCDF	1,2,3,4,6,7,8,9	66	—	—	ND	0.20	—	ND	0.20	—

^aND indicates not detected at concentration in excess of the detection limits.

Table 3. PCDDs/PCDFs in biological samples from patients.

Patient	Sample type	Concentrations of total PCDDs/PCDFs (minimum detectable quantity in parentheses in cases where none detected), ppt									
		TCDDs	TCDFs	PCDDs	PCDFs	HxCDDs	HxCDFs	HpCDDs	HpCDFs	OCDDs	OCDFs
B	Adipose	ND (10)	ND (10)	ND (20)	ND (20)	ND (30)	ND (30)	130	a	300	ND (50)
B	Blood	ND (1)	ND (1)	ND (5)	ND (5)	ND (7)	ND (7)	ND (6)	a	ND (5)	ND (5)
D	Adipose	ND (30)	ND (30)	ND (50)	ND (50)	ND (100)	ND (100)	250	ND (200)	600	ND (300)
C	Adipose	ND (10)	ND (10)	ND (20)	ND (20)	ND (30)	ND (30)	240	ND (150)	750	ND (80)
C	Blood	ND (2)	ND (2)	ND (5)	ND (5)	ND (7)	ND (7)	ND (10)	a	100	ND (5)
E.S. (control)	Adipose	ND (10)	ND (5)	ND (15)	ND (15)	ND (20)	ND (20)	200	ND (70)	800	ND (30)
H.E. (control)	Adipose	ND (10)	ND (10)	ND (20)	ND (20)	ND (30)	ND (30)	100	a	650	ND (100)

^aMajor interference from apparent chlorodiphenyl ether residue in sample.

Table 4. Occurrence of furan and dioxin isomers in adipose tissue.

Reported occurrence	Isomer	Controls (1-4) ^a				(Exposed)
		Patient 1, S	Patient 2, S	Patient 3, S	Patient 4, S	Patient 5, S
BSOB, Y ^c	2,3,7,8-F	ND(2ppt) ^b	4.1	ND(2ppt)	ND(2ppt)	ND(2ppt)
	2,3,7,8-D	8.3	7.2	6.0	3.7	11.6
	2,3,4,7,8-F	12.5	10.9	17.0	16.5	74.7
	1,2,3,7,8-D	13.8	10.3	8.2	7.5	15.0
BSOB, Y	1,2,3,4,7,8-F	11.4	9.3	13.0	22.9	149
BSOB, Y	1,2,3,6,7,8-F	5.6	5.8	8.8	15.4	112
	1,2,3,6,7,8-D	46.2	54.5	60.3	60.4	72.6
	1,2,3,7,8,9-D	7.4	7.5	7.4	6.8	7.3
BSOB	1,2,3,4,6,7,8-F	16.3	13.7	12.5	23.8	39.3
BSOB	1,2,3,4,7,8,9-F	ND	ND	19.6	20.6	25.9
	1,2,3,4,6,7,9-D	ND	ND	2.7	5.3	9.6
	1,2,3,4,6,7,8-D	95.8	39.4	119	93.1	209
	OCDF	nd(20)	nd(20)	1.2	1.5	1.6
	OCDD	534	593	695	586	690

^a Source of fatty tissue: (S) subcutaneous abdominal or buttock adipose tissue; (M) Intra-abdominal mesenteric adipose tissue.

^b ND: none detected at cited sensitivity.

^c Y = toxic furan isomers thought to have accounted for the Yusho rice oil toxicity; BSOB = isomers found in the exposed patient which were reported by Rappe (3) as being abundant in Binghamton State Office Building soot.

extended period of time. He had 8400 ppt of hepta- and octachlorinated dibenzodioxins and dibenzofurans with 7700 ppt hepta-CDFs, 200 ppt hepta-CDDs, and 500 ppt octa-CDDs. He also had the highest serum PCB values of the almost 500 patients in the original Binghamton medical surveillance program, ranging between 47 and 60 ppb. This patient had elevated serum cholesterol and triglycerides (up to 2200 in one instance) as well as hypertension, all of which have previously been reported as being related to PCB exposure. Of special interest in this patient is the correlation of the highest serum PCB level with the highest adipose tissue furan and dioxin levels within this group of patients. Also, the difficulty of estimating exposure to PCBs with such extensive serum lipid levels must be contrasted with the theoretical simplicity in which this question (that of excess serum lipids leading to increased serum PCB levels in a "spurious" or misleading fashion) is resolved by the fat biopsy, which is not complicated by high or low serum lipid levels leading to high or low PCB levels not truly reflective of true body burden. Upon extensive questioning, no other source of the dioxins or furans other than the State Office Building could be found. There was no history of phenoxy herbicide or chlorinated phenol use, no previous similar incidents, no municipal incinerators near his home and no excess ingestion of fish or known contaminated drinking water. At the time of manuscript preparation, analysis was still in progress to determine the source of the high furan, dioxin and PCB levels, although it is reasonable to conclude from the clinical history that some must have been from the Binghamton State Office Building.

Analysis of this patient's adipose tissue (23) showed that there was a close correlation between furan isomers in the fat and furan isomers in the soot from the contaminated building.

Of considerable interest to us was the finding of levels of hepta- and octachlorinated furans and dioxins usually

in the amount of several hundred parts per trillion but up to 2000 ppt in fat removed from patients during surgical procedures but with no known exposure to these compounds. Like the earlier finding of DDT and metabolites in human fat several decades ago, this suggests dioxin and furan contamination of the environment with ingestion by humans.

Discussion

This evaluation of a subset of patients being followed as private patients in an occupational medicine clinic setting demonstrates that PCB patterns suggestive of exposure to a given incident can provide evidence of exposure through a "fingerprint"-like pattern analysis or suggest lack of exposure to a given toxic compound by lack of such pattern.

Second, fat biopsies are simple outpatient procedures, easily tolerated, comparable to a dental visit. They provide a means to resolve the question of whether or not serum PCBs adequately reflect body burden or might be "erroneously" high due to increased lipid in the serum. Also, these chemicals move rapidly into blood and then from blood into adipose tissue, liver, kidney, lung and other target organs so that unless sequential blood values are obtained, ideally starting before an incident but certainly immediately after and with considerable frequency and regularity for many months afterwards, it is difficult to interpret one or several serum PCB levels. Also, at this time fat biopsies are the only practical way to measure in the low parts per trillion range probably current U.S. "background" levels, and above or soon at parts per quadrillion (ppq) level, for the various isomers of furans and dioxins, and hence to estimate body burden. Fat biopsy results here seem to correlate well with (clinically estimated) exposure and also point out the necessity for analysing for multiple isomers. We also discovered surprising high

levels of contamination of adipose tissue in some patients not known to have been exposed to dioxins and furans, especially with the 7- and 8-chlorinated compounds, about which so very little is known at this time with respect to toxicity or lack of toxicity in humans.

Third, electron microscopic lesions similar to those previously described in animals after feeding experiments with PCBs or dioxins or in humans after chlordecone (Kepone) poisoning were found in human hepatic parenchymal cells after dioxin, furan and PCB exposure. These lesions, especially the mitochondrial alterations, pleomorphism, branching, giant mitochondria, crystalline structures within mitochondria, and cristae mitochondriales aligned parallel to the mitochondrial long axis and stacked in arrays may constitute a portion of a characteristic pathognomonic entity, hence a second, after positive fat biopsy findings, biological marker for exposure to dioxins and related compounds in humans.

NOTE ADDED IN PROOF: Recent improvements in chemical techniques have led to an almost exact matching of furan isomers found in soot from the BSOP, and in fat from an exposed patient (23). Table 4 illustrates this correspondence.

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